EFFECTS OF X-IRRADIATION OF MICE EXPOSED IN UTERO DURING DIFFERENT STAGES OF EMBRYOLOGICAL DEVELOPMENT ON DURATION OF MATURE LIFE[1]

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In an earlier paper (Nash and Gowen, 1962) we have shown that postnatal growth, as measured by weight changes to 75 days, following x-ray irradiation *in utero*, was dependent on both the levels of irradiation and the embryological ages at the time of irradiation. The embryological ages in order of increasing sensitivity were 6½, 17½, 14½ and 10½ days. Growth was measured for the periods from mating to birth, and to 12, 26, 40, 60 and 75 days postnatal ages. Body weight response was found to be noticeably dependent upon the age at which observations were recorded. Treatments that produced significantly lowered body weights did not usually show maximum effects until 40 days after birth. Growth changes, expressed as continuing reductions of the body weights with days post-parturition, were most obvious at 75 days of age. The reductions were greatest for the 320 r-treated mice, the lowering of the growth rates being dose-dependent.

The data on which these observations were made have one common property; the mice were alive at the time when the observations were initiated. This results in the population under observation being biased toward greater viability. This limitation is intrinsic at whatever level of development the data on the irradiation effects are taken. To, in part, reduce this difficulty in evaluating the irradiation effects on the animal's subsequent well-being, several other characteristics, such as anatomical changes in young born dead, lifetime fecundity and fertility and various parts of the lifespan, have been utilized for measuring as yet unrecognized physiological changes in those exposed to irradiation. This paper deals with those changes, which may be immediate or long delayed, which affect lifespan after the beginning of the reproductive period.

Lifespan is a unique instrument in that it integrates the effects of external as well as internal agents influencing life processes. It covers both the short-term and long-term effects as well as the constitutional characteristics of those exposed. It has more variation, both innate and accidental, than other measures so differences between agents believed to affect life become harder to demonstrate. Yet it is in this characteristic most of us are interested.

Irradiation of the mammalian embryo exposes rapidly dividing cells during the intense process of organ formation and differentiation. It is a time when

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gene and chromosome changes would most likely result from single acute irradiation. Mitotic changes in single cells should grow to islands of tissue having functions different from those of the rest of the body complex or organ. The changes in the compatibility of the functioning units can influence those of the body as a whole and be expressed in changes in life's duration (Gowen, 1962).

MATERIALS AND METHODS

The embryos irradiated were obtained exclusively from first pregnancies in matings within and among three of our long-inbred strains of mice. After mating, the mothers marked for subsequent irradiation were examined daily for the presence of a vaginal plug. The appearance of this plug was considered the time of fertilization and the start of the events covered by gestation. Embryo ages were calculated from this point. All mice were considered to have mated during the same time at night, with 4 AM as the most likely point for the union of sperm and egg. Irradiations were begun at 4 PM; embryological ages at irradiation were set at $6\frac{1}{2}$, $10\frac{1}{2}$, $14\frac{1}{2}$ and $17\frac{1}{2}$ days. In a later series, irradiations of newborn young were indicated as of $19\frac{1}{2}$ days of age. These mice add breadth to the observations in that they alone are irradiated, whereas both mothers and young are treated at the earlier ages with the possibility that a maternal effect may be added to the direct effect of irradiation on the progeny.

The inbred females irradiated were of strains long under study and differentiation at the Genetics Laboratory of Iowa State University. The major criteria of differentiation have been relative resistance to several diseases of bacterial or virus origin, chemical poisons and radiant energy (Schott, 1932; Gowen and Schott, 1933a, 1933b; Hetzer, 1937; Gowen and Zelle, 1945; Gowen, 1955a, 1955b; Gowen and Stadler, 1956; Stadler and Gowen, 1957a, 1957b, 1957c; Gowen, 1960; Stadler and Gowen, 1961, 1963). The Committee on Mouse Nomenclature has designated the strains as BALB/Gw, K and S. The three strains cover a wide range of susceptibility to radiation. The BALB/Gw (hereafter called Ba) and K strains are relatively susceptible to acute irradiation, whereas the S is relatively resistant.

All progeny were weaned at 30 days of age and males separated from the females. At 75 days of age mice were individually mated to non-irradiated mice of the Z strain. This strain has an entirely different origin than the Ba, K or S. It is noted for regularity, frequency and size of litters. Previously unmated Z males or females were used in these matings. The treated animals were mated to Z mates throughout their lives. To have balanced numbers of mice tested for lifespan and reproductive performance, two males and two females were selected when possible from each treatment group. The after-mating lifespans of these treated animals start at the day of mating and run to the day of death. The full lifespan from birth of any mouse is determined by adding 75 days to the mating-to-death interval. If a litter did not contain two males and two females in each treatment group, an additional litter from another female, raised to 75 days, was used to obtain the additional mice to complete the cell, two each of treated males or females.

When a Z mate died before its treated partner it was replaced within a few days by another virgin Z mouse of an age between two and six months. All matings were retained for the lifetime of the treated mouse.

All matings were observed daily and the following information taken: (a) lifespan of each treated mouse, (b) total number of litters, (c) number of progeny within litters, (d) birth weights of individuals, (e) viability of mice within litters from birth to 21 days, and (f) any external anomalies which the mouse might carry.

Irradiation exposures were chosen to cover the range from none to 320 roentgens, the highest exposure that would allow survival of at least some of the embryos in the embryonic age series. There were five exposures, 0, 20, 80, 160 and 320 r. Five stages in embryological development were treated with the chosen dosages of irradiation. The embryos treated were either males or females of which samples of two each were chosen. The genotypes were divided into nine groups; three represented the pure inbred strains, six their possible hybrids made reciprocally. The design is a factorial in which the treatments are regarded as fixed points. Unfortunately, of the mice treated with 320 r at 6½, with 160 r and 320 r at 10½, none survived for lifespan determinations, and of the 320 r at 14½ embryos only three progeny remained for determining lifespans for this group. In consequence, the design as a whole loses its orthogonality. However, the greater portions of the data may be arranged in different designs to retain the orthogonal principle even though they require separate analyses.

A General Electric Maxitron operated at 250 pkv, 30 ma with 0.25 mm. Cu + 1 mm. Al filtration at a distance of 50 cm. from anode to mid-mouse, dose rate 133 r/minute, furnished the x-rays for the treatments. Dose was measured in air by a rate meter at a position corresponding to that of the mid-mouse. Pregnant mice were exposed to single doses of whole-body irradiation in circular wooden containers 6½ inches in diameter and 1 inch deep. The base of the container was ¼ × ¼ inch hardware cloth. The top was covered with two layers of cellophane. Newborn mice of a whole litter were irradiated whole body in small plastic trays and then immediately returned to their mothers. Following custom, all roentgen dose measurements were taken in air at the mid-position the mouse would occupy when irradiated. These dosages do not represent the energies actually reaching different organs of the exposed mouse. They are subject to different adjustments dependent on the particular tissue and absorptions in different body tissues having characteristic atomic densities. If, for instance, the roentgen dose of 250 pkv, 30 ma filtered through 0.25 mm. Cu and 1 mm. Al to an adult female mouse is measured as 1 r, the dose when absorbed in passing through bone and back muscles to the ovaries becomes about 0.85 r at the position of the ovaries. The reader should take these effects into account. They will be discussed at some later date.

The mice throughout were kept in a well ventilated room, in which the environment and management were relatively constant. Compressed food pellets and water were before the mice at all times.

Frequency Distributions of Post-75-day Lifespans as Affected by Sex Treated, Previous Early Irradiation Exposures While in utero or at Birth, Embryological Ages at Treatments, and Genotypes of Strains Treated

Frequency distributions, even though based on rather small numbers, assist in evaluating the effectiveness and mode of action of the different treatment agents on the adult lifespans of those exposed. These distributions, for treated males and for treated females, are shown at the top of Figure 1.

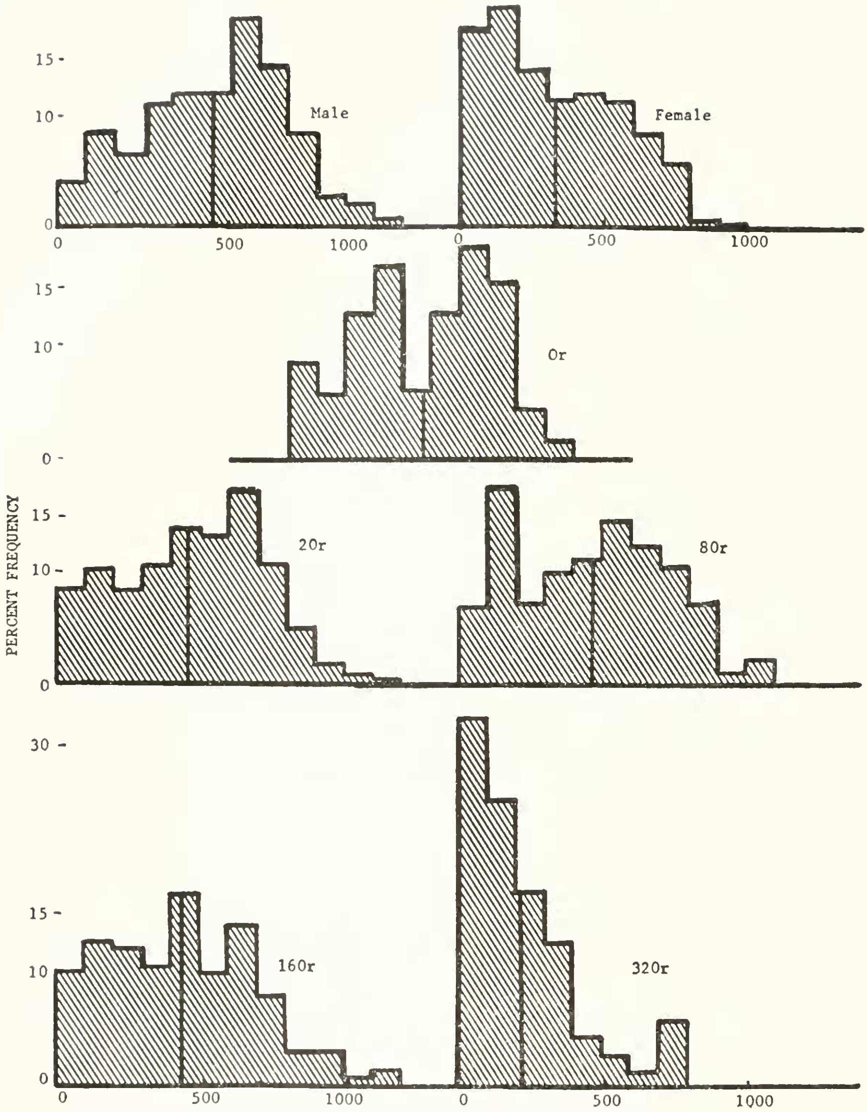

Figure 1. Frequency distributions of adult lifespans of male treated or female treated mice are shown at the top of the chart. The effects of different x-ray dosages 0 r, 20 r, 80 r, 160 r, 320 r are shown in lower part of chart.

The lower three-fourths of Figure 1 gives the frequency distributions for length of life from mating to death of those mice unexposed to x-irradiation, exposed to 20 r, to 80 r, to 160 r or to 320 r at some time from conception to those which are newborn. The viewer should adjust any conclusions derived from these frequencies to the fact that no mice survived to 94 days post-conception in those treatments previously specified.

The distribution of adult male lives shows a longer mean life than that for the females. The forms of the distributions are also different. The male histogram has its modal lifespan at a higher age than that for the mean. The histogram for the female is less symmetrical than that for the males, with the modal value less than that for the treated males. Skewness is positive in contrast to that for the males being negative. The frequency distribution for the females departs significantly from normality.

The five histograms for mice untreated with x-irradiation, 0 r, and those

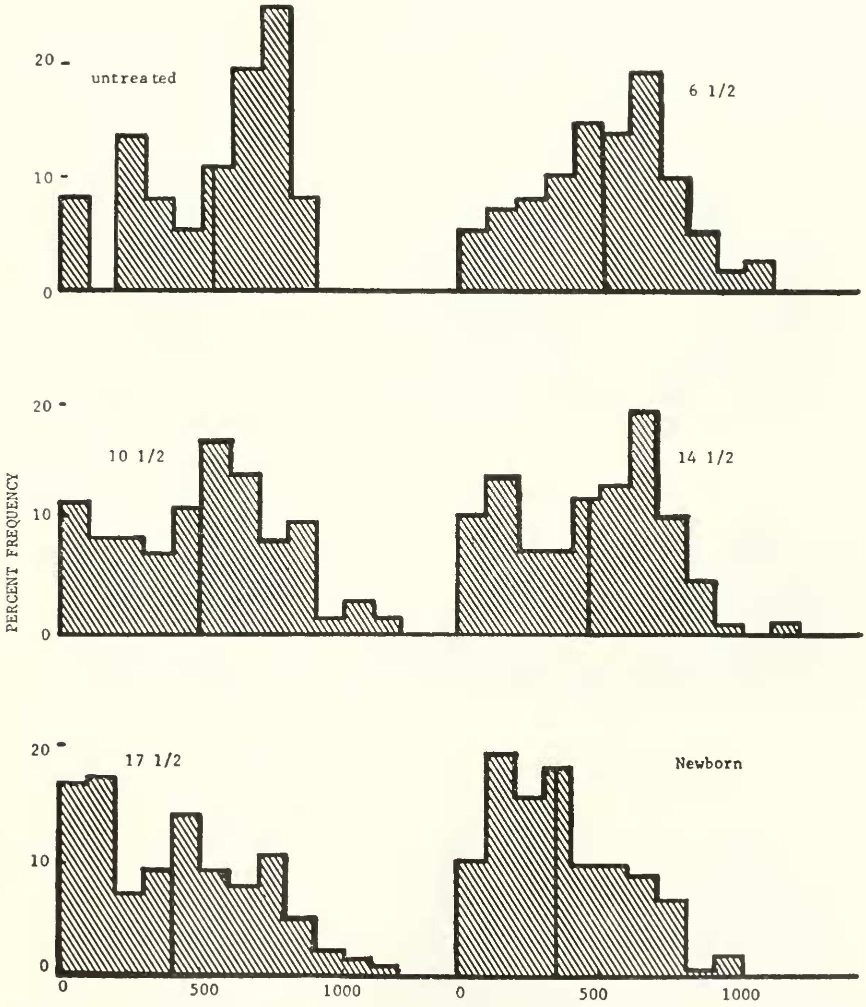

FIGURE 2. Histograms showing frequencies of adult lifespans of mice exposed to x-irradiation at different periods in embryological development. The charts show the per cent frequencies of those living within each 100-day age group from the date of mating, 75 days of age, to the last death in the populations.

exposed to 20 r, 80 r, 160 r and 320 r are presented in the lower part of Figure 1. These histograms show noticeable differences in form and, in consequence, in their means, modes, variances and skewnesses. The most striking difference is the J-shaped distribution of the 320 r treated mice. Deaths occur earlier and more rapidly than in the other distributions for the lower irradiation dosages. This 320 r histogram presumably would be even more extreme if proper account could be taken of all the classes of mice which were treated with 320 r. Death *in utero* from the 160 r at 10½ days also modified the 160 r histogram in removing these mice from the recorded observations. Later analyses will attempt to evaluate these effects.

Figure 2 shows the distributions of the post-75-day deaths by successive 100-day age intervals. The untreated mice die most frequently at the higher ages. The distribution for the mice exposed *in utero* at 6½ days shows similar but less extreme features. However, the skewness may be partly illusory, for, as indicated, the

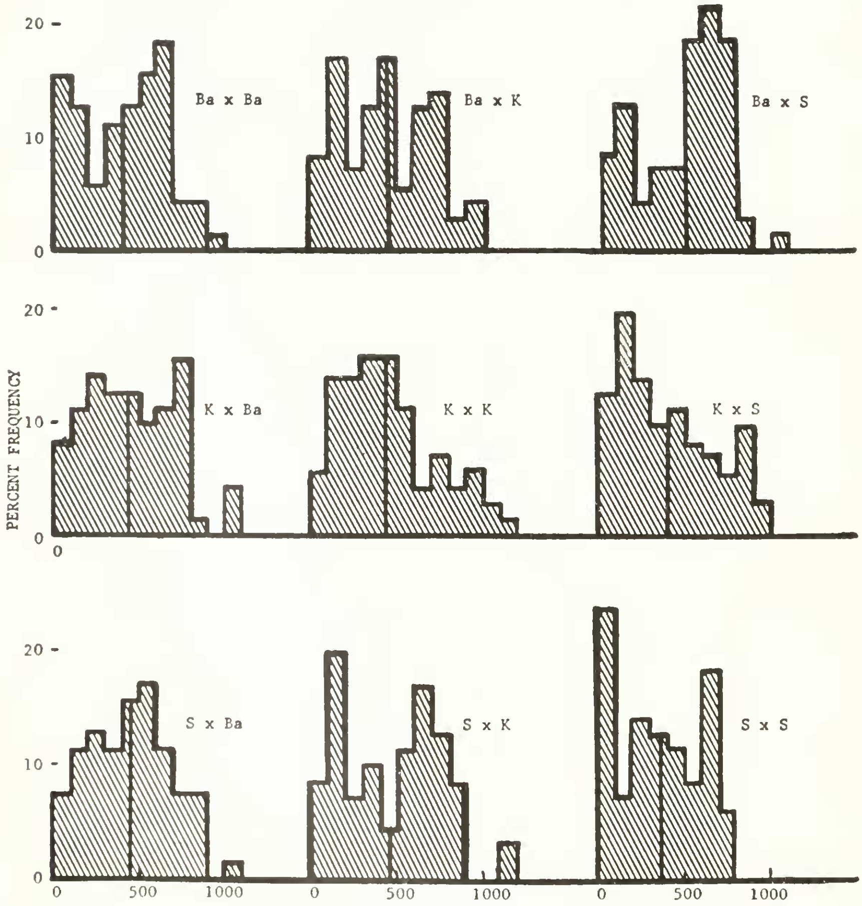

FIGURE 3. Histograms for the three inbred strains and their possible hybrids, showing the percentage frequencies of deaths for each 100-day interval after mating.

distribution does not contain the 320-r irradiated mice. The same considerations apply to the histograms for 10½-day and 14½-day mice. The 17½-day and newborn data are orthogonal since they include all treatments. They are different from the control mice in having their higher frequencies of deaths during the earlier ages. The adult lifespans are positively skewed as contrasted with the negatively skewed characteristics of the un-x-rayed population of mice.

Similar histograms have been drawn for the populations of mice of the 9 different genotypes. The data are also arranged to show the differences between the pure inbred strains and their reciprocal hybrids (Fig. 3). As may be expected the histograms vary widely. The inbreds Ba × Ba and S × S show some tendency to U-shaped distributions although this form is unlikely on both biological and theoretical grounds. The S × S distribution is furthermore not in conformity with our other extensive data, in that this strain ordinarily shows much higher resistance to irradiation, lifespans of 800 days not being uncommon when the irradiation occurs later in life, 46 days from birth, than in the embryonic period. The data sample is also anomalous in having the high frequency of deaths in the 100 days immediately after mating. The ranges in lifespans of the other genotypes are fairly comparable to our earlier data. The histograms show examples of both positive and negative skewness.

The relations between the percentage frequencies of death on the different 100-day age intervals after mating for inbred vs. hybrid genotypes are shown for the combined data at the top of Figure 4. The individual comparisons between the three inbreds and their individual crosses and reciprocals are shown in nine histograms in the lower three-fourths of the chart. The hybrids' average lifespans are longer than the inbreds'. However, some inbreds live as long as any hybrids. The reduction in the average comes in fewer animals living over 700 to 1200 days. There is a tendency toward rectangular distributions of the death frequencies to 800 days from the mating date.

Mature Lifespans Distributed According to Sex, X-ray Exposure, Embryological Age, Strain, Inbred, Hybrid or Reciprocal Crosses

The constants for the frequency distributions (Figures 1 including 4), presented in Table I analyze the characteristics of the different curves and allow an over-all estimation of the frequency curve types in Pearson's systematic treatment of the problem, from the values of β_1 and β_2. Unfortunately, the numbers of individuals in the finer divisions of the populations are frequently smaller than desirable.

Sex effects on survival are noticeable in the means and medians of the mature lifespans. The mean and median and its related function, the mode, establish the curve position on the axis. If the median is the larger, as for the male mice, the curve ranges from nearly symmetrical to negatively skewed, as shown by the g_1 values. The β_2 constant estimates whether the curve is more flat-topped or more peaked, the kurtosis, than the so-called "normal" curve. The significance of the deviations is shown by the t values for g_1 and g_2. The males show greater durations of life after maturity, somewhat more variation in survival time; their frequency distribution is negatively skewed and has significant kurtosis. The female lives in the maturity period are shorter, the median point in their deaths is earlier and the variations are less than those of the males. The distribution of their life-

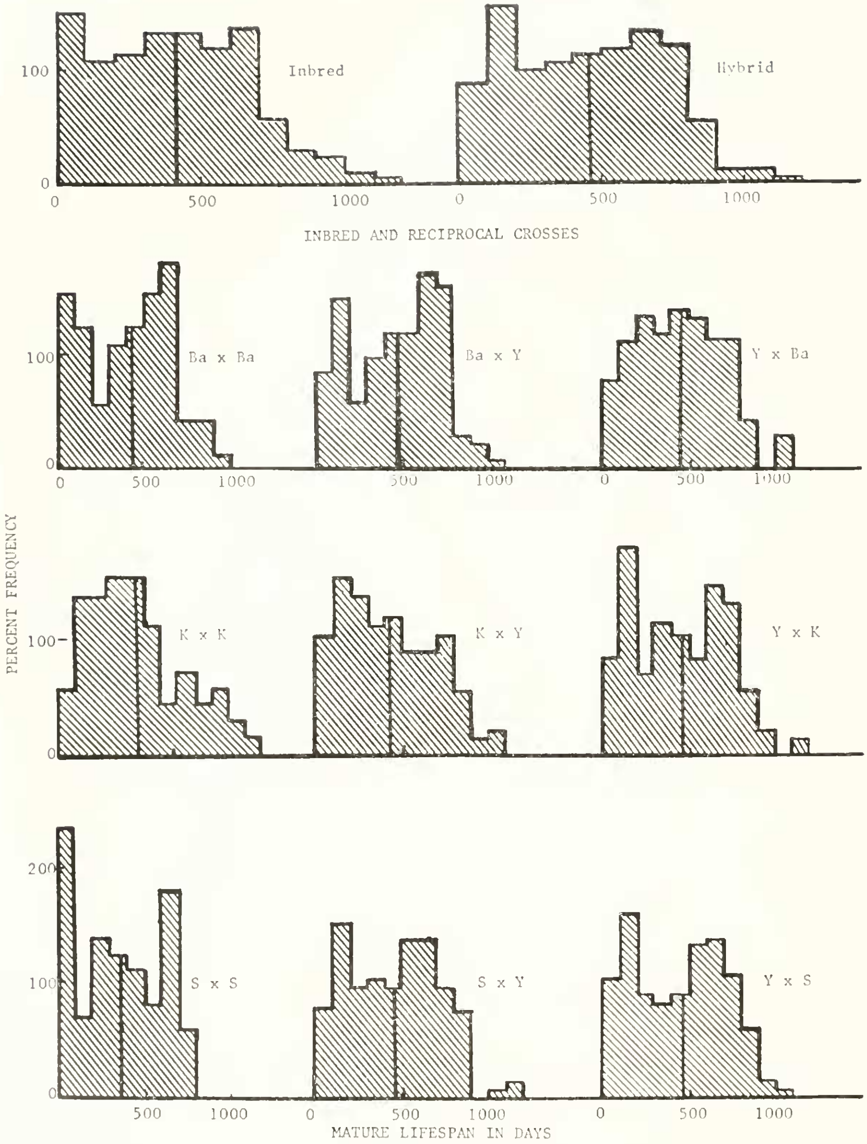

FIGURE 4. Percentage frequencies of deaths plotted on the different 100-day age intervals after mating for combined inbred mice of three strains and their possible hybrids are shown at the top of the chart. The individual inbreds and their possible crosses are plotted in the lower nine histograms, inbreds on the left. Crosses for the males of the same inbred in the middle, *Y* representing the averages of the two crosses. On the right the constant parent is the female inbred of the strain at the left.

TABLE I

Constants for frequency distributions

	Mean	Median	Standard deviation	β_1	β_2	t for g_1	t for g_2
Sexes							
Males	540 ± 14	568	249 ± 8	0.0178	2.405	1.0	2.2 (1)
Females	332 ± 12	299	224 ± 6	0.1627	2.095	3.0 (4)	3.3 (4)
X-ray exposure							
0 r	479 ± 29	505	243 ± 15	0.0416	2.043	0.7	1.7
20 r	474 ± 19	487	250 ± 11	0.0000	2.308	0.0	1.8
80 r	465 ± 19	468	257 ± 10	0.0078	1.991	0.5	2.7 (3)
160 r	438 ± 21	424	257 ± 14	0.0942	2.621	1.5	0.9
320 r	223 ± 22	157	189 ± 19	1.6021	3.946	4.6 (4)	2.0 (1)
Embryological age							
untreated	545 ± 40	601	242 ± 23	0.4270	2.328	1.7	0.8
6½ days	507 ± 23	519	240 ± 14	0.0086	2.512	0.4	1.0
10½ days	493 ± 32	518	276 ± 18	0.0002	2.163	0.5	1.4
14½ days	461 ± 24	493	254 ± 13	0.0046	2.213	0.3	1.7
17½ days	404 ± 23	390	280 ± 12	0.1550	2.112	2.0 (1)	2.2 (1)
Newborn	360 ± 16	337	220 ± 10	0.2391	2.487	2.7 (3)	1.4
Strains							
Ba × Ba	414 ± 29	421	246 ± 14	0.0017	1.922	0.1	1.9
Ba × K	448 ± 31	428	261 ± 15	0.0091	1.933	0.3	1.9
Ba × S	491 ± 29	551	245 ± 15	0.1864	2.131	1.5	1.5
K × Ba	456 ± 31	433	260 ± 18	0.0350	2.304	0.7	1.2
K × K	451 ± 32	404	274 ± 21	0.3833	2.718	2.2 (1)	0.4
K × S	397 ± 31	332	262 ± 15	0.2189	1.983	1.7	1.8
S × Ba	445 ± 28	435	238 ± 15	0.0112	2.211	0.4	1.4
S × K	464 ± 33	514	279 ± 17	0.0159	2.046	0.5	1.7
S × S	359 ± 27	332	231 ± 12	0.0170	1.818	0.5	2.1
Inbreds	407 ± 17	398	254 ± 11	0.1146	2.492	2.1 (1)	1.5
Hybrids	450 ± 12	448	260 ± 6	0.0103	2.035	0.9	4.1 (4)
Reciprocal crosses							
Ba × Y	470 ± 21	499	254 ± 10	0.0250	1.958	0.8	2.5 (2)
Y × Ba	451 ± 21	435	250 ± 12	0.0250	2.290	0.8	1.7
K × Y	427 ± 22	390	263 ± 11	0.1026	2.099	1.6	2.2 (2)
Y × K	456 ± 23	448	270 ± 11	0.0140	2.011	0.6	2.4 (2)
S × Y	454 ± 22	463	260 ± 12	0.0186	2.163	0.7	2.1 (2)
Y × S	444 ± 22	461	258 ± 10	0.0006	1.802	0.1	3.0 (4)

Probability levels (1) = 0.05, (2) = 0.025, (3) = 0.01, (4) = 0.005 or less.

spans is positively skewed, the kurtosis being evident. Both skewness and kurtosis are significantly different from those of the "normal" curve.

The frequency curves for other subdivisions of the data show lowering of the mature lifespans with increasing dosages of the x-ray energy as well as differences between its effects in the different stages in the embryological life when the radiation was received. Inbred mice showed shorter durations of life than hybrids between the strains, and both inbreds and hybrids showed variations dependent on their origins. Negative and positive skewness was observed in the curve forms. Save for one case for mice treated with 320 r, the kurtosis was uniformly negative, reaching significance in 11 out of the 30 distributions.

The differences exerted by the different variables show that analyses of the effects on the mature lives of the preadolescent mice must be analyzed separately for within sex, dosages and embryological ages of treatment.

INFLUENCE OF SEX, X-RAY DOSAGES AND EMBRYOLOGICAL AGES WHEN THE IRRADIATIONS WERE RECEIVED ON MATURE LIFESPAN

Table II presents means and standard deviations for the lengths of the later lifespans distributed for embryological age at irradiation, dose of x-ray exposure and sex of the parent treated. The mean day survival is followed by its standard deviation. The standard deviations of the distributions of the raw data follow.

In every instance the after-mating lifespans of the males are noticeably longer than those of the females whatever may have been the time at which the developing embryos were irradiated, or the amount of irradiation to which they were exposed. This major factor in these lifespan differences, in consequence, is not attributable to the irradiations or the embryological ages at which irradiation occurred. They seem to turn on innate biological differences in the risk of death to the parent in carrying on reproduction. This risk is much greater to the females than to the males. The results support our earlier work in showing that pregnancies spaced at short intervals result in large reductions in the lifespans of the females, whereas the lifespans of the males are nearly unaffected (Gowen, 1960).

Irradiations with 20 and 80 r occurring at the different *in utero* periods showed little effect on the difference in life of the sexes, the females for the same treatments being about 0.6 those of the similarly treated males. A lengthening of the female lifespan occurred when the female was treated with 80 r as a newborn.

MATING-TO-DEATH SURVIVAL FOR MICE IRRADIATED IN UTERO IN RELATION TO DOSE RECEIVED

Figure 5 plots the mean after-mating lifespans of male and female mice distributed within the embryological ages at which the irradiations were given and by the dosage of irradiation they received. The curves for the intrauterine irradiated mice were similar in form. Sex differences in the lengths of the lifespans were marked. Doses to and including 160 r of acute irradiation caused a 72-day loss to males and a 119-day loss to females in their average durations of life, 11% and 27%, respectively, of their expected survivals after mating. The declines in the adult lifespans were irregular but in general followed trends which approach linearity. The reductions in life duration occurred even though the animals had 75 days in which to recover before measurements of the irradiation effects were begun. The groups were selected groups which had eliminated the rapidly fatal irradiation effects.

After the 160-roentgen dosages the effects on later life survival became more severe. Lifespans for mice exposed to 320 r intrauterine were reduced to 43% and 30% of those enjoyed by untreated male and female mice, respectively.

Figure 5 separates the effect of irradiations at different periods in the uterine development. The durations of adult life of males exposed at $6\frac{1}{2}$, $10\frac{1}{2}$, $14\frac{1}{2}$ and $17\frac{1}{2}$

TABLE II

Mean days and standard errors mating to death

Embryological age at irradiation	Irradiation dose	Sex treated	Mean	Standard deviation
Untreated	0 r	Male (M)	646 ± 49	208
		Female (F)	443 ± 57	242
6½ days	20 r	M	597 ± 55	233
		F	373 ± 56	237
	80 r	M	637 ± 55	234
		F	383 ± 54	228
	160 r	M	576 ± 58	246
		F	472 ± 34	143
10½	20 r	M	616 ± 47	201
		F	368 ± 63	268
	80 r	M	586 ± 70	297
		F	401 ± 63	269
14½ days	20 r	M	620 ± 33	141
		F	414 ± 57	244
	80 r	M	590 ± 48	210
		F	313 ± 49	206
	160 r	M	596 ± 60	256
		F	234 ± 43	184
	320 r	M	254	
		F	35	
17½ days	20 r	M	668 ± 55	232
		F	359 ± 49	210
	80 r	M	591 ± 63	266
		F	363 ± 50	212
	160 r	M	551 ± 65	275
		F	267 ± 48	202
	320 r	M	284 ± 61	259
		F	150 ± 39	165
Newborn	20 r	M	447 ± 41	174
		F	275 ± 51	216
	80 r	M	440 ± 50	211
		F	350 ± 50	213
	160 r	M	466 ± 62	263
		F	335 ± 49	209
	320 r	M	289 ± 35	151
		F	169 ± 31	133
Newborn	0 r	M	518 ± 52	220
		F	309 ± 46	194

days of uterine development are quite comparable. The lifespans for males treated as newborns are shorter than expected for the 0, 20, 80 and 160 r treatments. Females irradiated at 14½ days uterine development with 160 and 320 r had their durations of life noticeably reduced. Newborn females either untreated or exposed to 20 r showed noticeably shorter lifespans than expected. No explanation is known which will account for this difference. The newborn group was selected for study some time after the other treatment groups, but the mice in the group were, so far as known, similar to the others previously treated with irradiation when they were chosen.

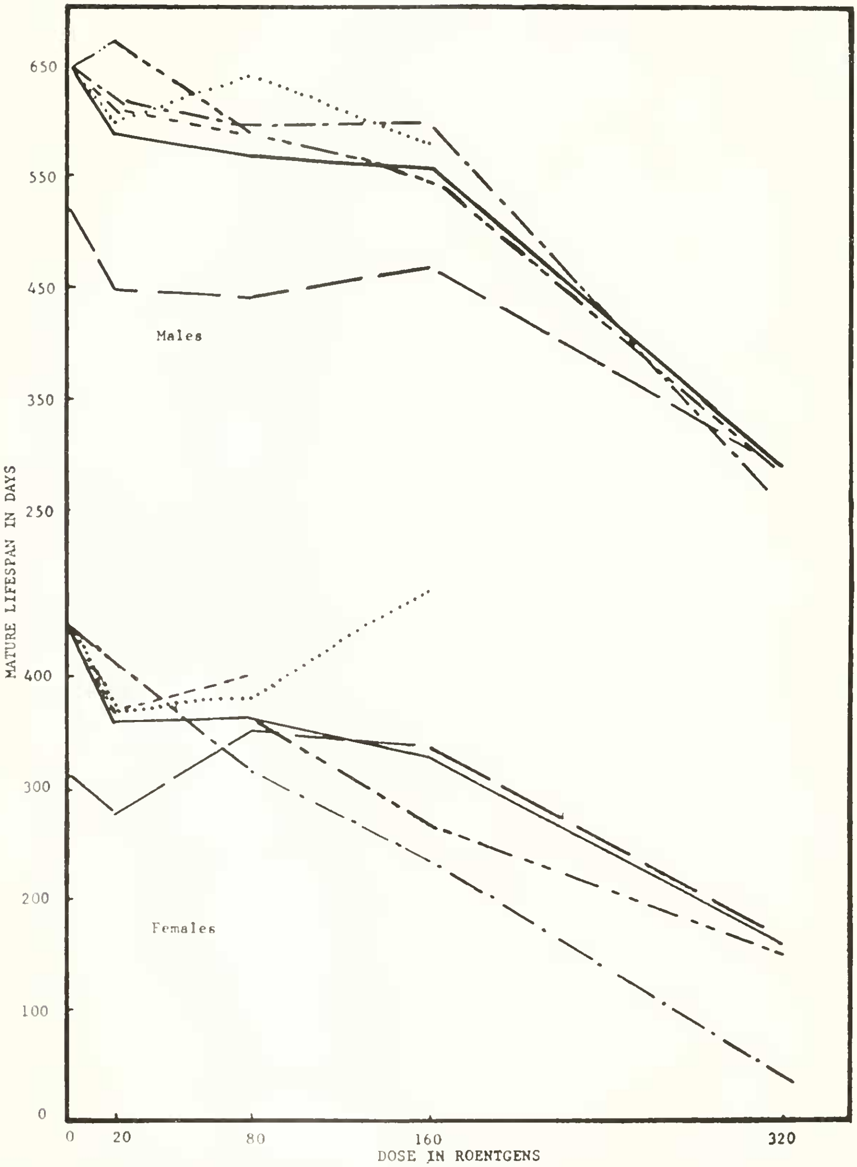

FIGURE 5. Mature lifespans of mice treated *in utero* with 0, 20, 80, 160 or 320 roentgens and plotted for the given embryological ages when the irradiations were absorbed. Embryological ages in days at treatment were designated by the following lines: 6½ ....; 10½ - - -; 14½ — - —; 17½— - - - —; newborn mice — —, and untreated ——.

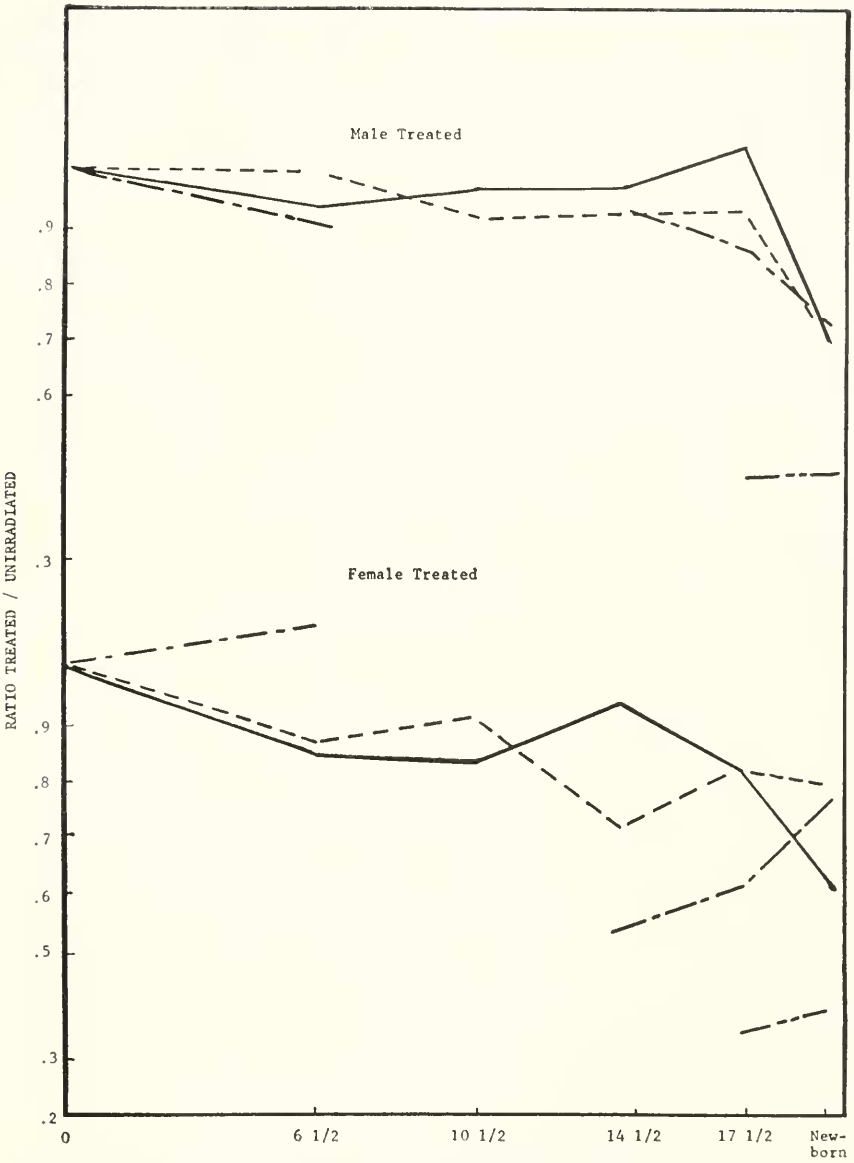

FIGURE 6. Lapsed days of life between mating to death of mice exposed at different embryological ages to irradiations of given dosages divided by the survivals of the corresponding unexposed mice. The dose of irradiation is indicated as follows: 20 r —; 80 r - - - -; 160 r — - —; 320 r — - - —.

The lower lifespans of the females are evident in all dosages and embryological ages and strains. For the *in utero* treated mice the average unirradiated females survived for only 69% of the mean male lifespan. This male-to-female difference is attributed to the hazards which differentially affect the physiological processes which surround reproductive functions. If the average female life under the different types of irradiation is adjusted for this difference to what would be expected for the male, the female still retains more susceptibility to irradiation than the male. Considering the available data for the dosages taken separately, the 20 r females had 85% of the mature mean lifespan observed for the untreated females, the males 96.7% that of the untreated males. Corresponding comparisons for the other dosages yield for 80 r, 82 vs. 93; 160 r, 73 vs. 89; and 320 r, 30 vs. 43% for females vs. males, respectively.

Comparisons of the sensitivity of the factors which make for longevity in embryos at different growth stages when irradiated are made difficult because all mice exposed to 320 r at 6½, 10½, as well as those in the 160 r, 10½-day embryo age groups, failed to reach the mating age. Only two males and one female lived beyond the 75-day date for mating in the 320 r 14½-day treatment group. These mice survived 31, 478 and 35 days following mating. While these data confirm the sensitivity of these mice to irradiation at these dosages and stages, they failed to give a group by which the effects of irradiation at these dosages and embryological stages may be determined in the reproductive and senescence periods of life. The two males and one female in the 320 r, 14½ embryological age group indicate that the life shortening would be severe.

Figure 6, as the companion graph to Figure 5, brings out the contribution of embryological age to sensitivity toward high energy irradiation. The results are determined as the ratios of mature lifespans of irradiated males or females to those of the unexposed mice of like sex. They are plotted on the embryological ages when the irradiations were received. Full data on the separate sexes were obtained on the 0 r, 20 r, and 80 r for each embryological age. Curves for the 160 and 320 r are broken for the reasons previously indicated.

Unirradiated mice on the average had longer lifespans between mating and death than those which were irradiated. For mice irradiated with 20 r and 80 r the mature lifespans change but little over the span during which embryological x-ray exposures occurred. The female adult lifespans were depressed somewhat more than those of the males. Dosages of 160 r and above tended to further reduce the days the mice survived. When the irradiations reach 160 r at 10½ days embryological development or 320 r at 6½, 10½ days, the development of all mice was interrupted before they reached the mating age. That these early uterine irradiations would have caused severe damage to after-mating life was shown by the fact that the two males and one female which did survive this dose at 14½ days died early, 31 and 478 days for the males and 35 days for the female.

REPRODUCTIVE AND SENESCENT LIFESPANS OF MALE AND FEMALE MICE FOLLOWING IRRADIATIONS OF DIFFERENT DOSAGES RECEIVED AT DIFFERENT PERIODS OF EMBRYOLOGICAL DEVELOPMENT

The mean reproductive lifespans of the males were noticeably longer than those of the females in these data. The comparative differences are brought out in Figure 7.

Figure 7 shows a rather constant relation between the lifespans of female to male mice for those treated with x-rays of 20 and 80 r over the different embryological periods. The severity of the effects at 160 and 320 r for the 6½, 10½ and 14½

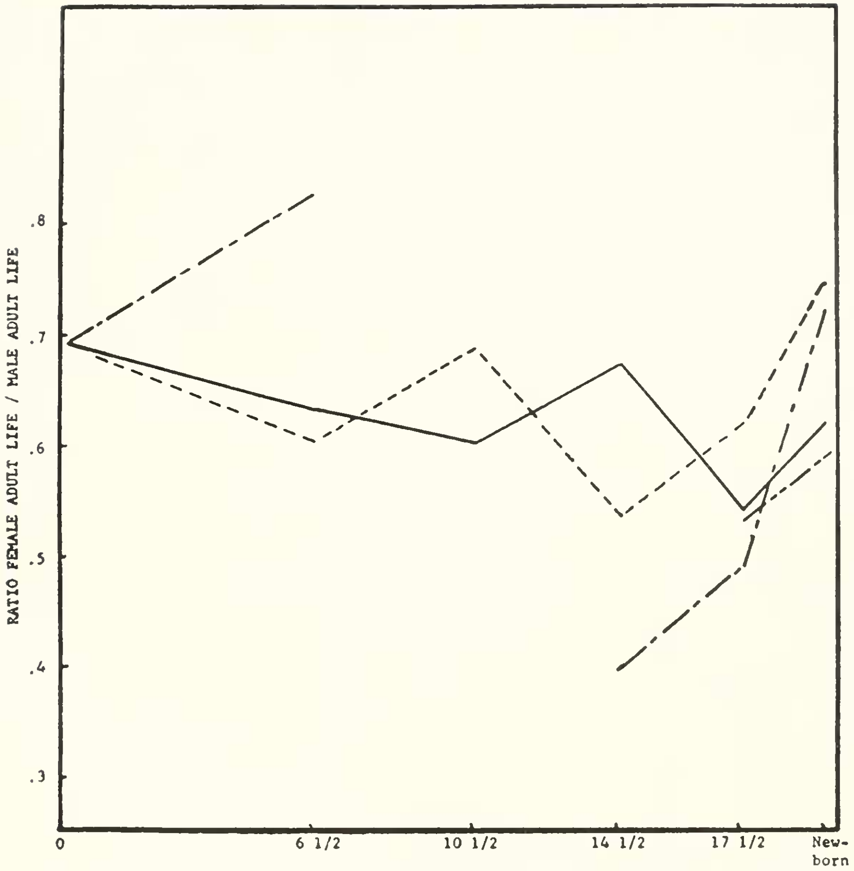

Figure 7. Ratios of the mature lifespans of females to those of males for the same irradiation exposures, plotted against the embryological ages when treatment took place. Radiation dosages: 20 r —; 80 r - - - -; 160 r — - —; and 320 r — - - —.

periods of gestation indicate that for higher dosages of x-ray, differences in the lethal or semilethal capacities do appear as embryological development unfolds. The individuals which survive to adulthood bear these unseen effects. The effects were more severe on the female than on the male mice.

Analyses of the Variations Observed in Mature Lifespans

In these data the mature lifespans are regarded as dependent on measurable events occurring some 75 or more days earlier, in the interim conception-to-birth, than when the mature lives took their origin. These events are the characteristics of the basic mouse genotypes and sexes, which compose the mouse population, acted upon by the different dosages of irradiation occuring at specific embryological stages of development in the life cycle. In symbols the equation describing the relationship is of the familiar linear type. The plan of the study as indicated earlier

TABLE III

Duration of mature life as related to exposure to high energy irradiations at different stages of embryonic development

Source of variation	Embryological age (U) in days at exposure to irradiation														
	6½			10½			14½			17½			Newborn		
	df	M.S.	*P*	df	M.S.	*P*	df	M.S.	*P*	df	M.S.	*P*	df	M.S.	*P*
Dose (T)	3	230		2	325		3	1261	(3)	4	6134	(4)	4	2066	(4)
Strain (G)	8	227		8	435		8	1375	(4)	8	895	(2)	8	1640	(4)
T × G	24	624		16	995	(1)	24	564		32	846	(4)	32	808	(4)
Sex treated (F)	1	13718	(4)	1	12171	(4)	1	24748	(4)	1	24098	(4)	1	9344	(4)
T × F	3	398		2	96		3	506		4	430		4	194	
G × F	8	551		8	797		8	292		8	986	(2)	8	226	
GTF	24	547		16	514		24	444		32	403		32	274	
Unaccounted for (E)	72	469		54	548		72	346		90	383		90	216	
Dosage classes	0, 20, 80, 160 r			0, 20, 80 r			0, 20, 80, 160 r			0, 20, 80, 160, 320 r			0, 20, 80, 160, 320 r		

df = degrees of freedom. M.S. mean square ÷ 100. *P* probability, level of significance utilizing the unaccounted for variance (*E*) as the error term for fixed variates. *P* probability levels, 1 = 0.05, 2 = 0.025, 3 = 0.01, 4 = 0.005 or less.

was orthogonal. The biological nature of the results made the results lack orthogonality at several points. However, there are several desirable orthogonal arrangements of the data which give useful information on the effects of the different independent variables on lifespan. We shall present the two analyses which have proved most informative.

The variance constants of Tables III and IV allow evaluations of the after-effects on mature life processes of mice irradiated some 75 days or one-eighth of their expected lifespan earlier. The mice in these studies have withstood the

TABLE IV

Duration of mature life as influenced by the embryological age when the mice were irradiated with different dosages of high energy x-rays

Source of variation	Dose of irradiation in roentgens											
	20 r			80 r			160 r			320 r		
	df	M.S.	*P*	df	M.S.	*P*	df	M.S.	*P*	df	M.S.	*P*
Embryological age (U)	4	1487	(4)	4	724		3	1255	(2)	1	25	
Strain (G)	8	942	(3)	8	539		8	1110	(4)	8	883	(4)
U × G	32	698	(4)	32	791		24	1044	(4)	8	497	(1)
Sex treated (F)	1	24123	(4)	1	19245	(4)	1	17308	(4)	1	2892	(4)
UF	4	235		4	724		3	1400	(2)	1	7	
GF	8	661		8	662		8	321		8	457	
GUF	32	524		32	698		24	302		8	76	
Unaccounted for (E)	90	328		90	455		72	360		36	210	
Embryological age classes	6½, 10½, 14½, 17½, Newborn			6½, 10½, 14½, 17½, Newborn			6½, 14½, 17½, Newborn			17½ Newborn		

Symbols as in Table II.

physiological consequences over the earlier parts of their lives following the acute embryonic irradiation of different dosages. They are to that extent a group, selected by nature as being resistant. The irradiation effects observed are long-time effects.

The first arrangement of the data (Table III) is analyzed within constant embryological ages, when the irradiations occurred, and the numbers of mice for the different dosages. These data form an orthogonal design. The x-ray dosages given the treated groups are shown at the bottom of Table III. In the 6½ embryological age treatments there were 4 dosages of x-rays, 0, 20, 80, and 160 r, which were complete in having all cells filled in the factorial design. In the 10½-day embryological age group there were 3 dosages which had equal numbers of mice throughout, 0, 20 and 80 r, etc.

Table III shows that differences in irradiation doses, in the strains of mice exposed to the irradiations and in sexes of the individuals treated, all influenced the duration of mature life when the irradiations came in the latter half of pregnancy or in the newborn. Throughout, the greatest influence on the lifespan by a wide margin was the sex of the mouse. However, none of the sex interactions with dose or strains showed significant deviations.

The fact that the variances for dose of irradiation and for strains were comparable to the random variance (E) in the 6½ and 10½ embryological age treatments is not to be taken as indicating these variables have no effect in causing life shortening for the full possible range of the irradiation dose treatments. The 320 r dose so severely affected lifespan as to cause all those exposed to die early. The limitation of studying only after-mating lifespan prevented the irradiations from showing their full effects, as the severely injured mice all die early, *in utero* or shortly after exposure. Similarly, dosages of 160 r at 10½ days of embryonic age also resulted in the early deaths of all the progeny. Actually the irradiation treatments at the 10½-day age were probably the most significant to over-all life. The severity of the effects prevented them from showing in the mature aged group. The interesting fact which comes from these comparisons is that the mice can take the lower dosages at these embryological ages and yet not be affected significantly in their later lifespans, even though higher dosages are so toxic.

The data on the mice treated at 10½ days were self-curtailed as mice exposed to higher dosages than 80 r all died before they reached breeding age. Those treated with 80 r showed less than a 10% drop in lifespan over those which were untreated. The mice treated at 6½ days showed little dose effect. For 80 r there was a 6% and for 160 r only a 4% drop in lifespan. These facts suggest that the dosage effects *in utero* on life's duration after reaching adulthood would be small in the 20 and 80 r range. This point was tested by analyzing the data on the 14½, 17½ and newborn treated mice restricted to those exposed to 0, 20 and 80 r x-ray exposures. These analyses show that these dosages had only statistically insignificant effects although they each lowered the lifespans somewhat, 17%, 14% and 3%, respectively, for the 80 r treatments of the 14½, 17½ and newborn mice.

The data may be arranged to quantitate the effects of treatments at the different embryological ages when the dosages of irradiation are fixed at 20, 80, 160 and 320 r. These data are presented in Table IV.

The mean squares of Table IV show intrauterine treatments reduced significantly the durations of mature life in two of the four dosage treatments, 20 r and 160 r. Examination of the differences from which these variances were calculated shows that the observed significance values depend on the fact that the newborn mice, both untreated and x-ray irradiated, had lower average lifespans than the mice treated *in utero*. Why this should be is not clear. The mothers of these newborn mice were bred and randomly chosen from the same strains and in the same way as those bearing the mice exposed *in utero*. However, omitting this group from the calculations the results show that there were no real differences among mice treated at the different developmental stages, 6½, 10½, 14½ and 17½ days with 20 roentgens. If anything, their mature lifespans were increased.

Mice exposed to 160 r, if males, had their lives reduced in length 8% to 15%. If females, a gain in lifespan of 4% was noted if the mice were treated at 6½ days. A loss of 18% of the control lifespan occurred in the mice treated at 14½ days of embryological age with 160 r. The effects of the x-ray exposure to 160 r at embryological ages 6½, 14½ and 17½ days were significant even when the newborn mice were not considered in the analysis.

CONTRIBUTIONS OF THE DIFFERENT COMPONENTS TO VARIANCE

In the following interpretation it is assumed that the variations in lifespan are due to components acting additively. Each component is regarded as fixed, as contrasted with the assumption of randomness, as it is doubtful if any factor which is subdivided into less than 10 to 15 parts, even though selected at random, can really be considered as random in the sense of representing the whole biological population of a species or even the laboratory mouse. The considerations lead to the general equation portraying contributions of the variables influencing lifespan, Y, as

$$Y_{ijkl} = u + g_i + t_j + (gt)_{ij} + f_k + (fg)_{ik} + (ft)_{jk} + (fgt)_{ijk} + e_{ijkl}$$

where $u =$ the overall mean; $i = 1, 2, \ldots, 9$, the inheritance types; $j = 1, 2, 3$, (or 4 or 5), quantities of irradiation; and $k = 1, 2$, the sexes exposed to irradiation. The strain differences are considered as partial measures of the genotype effects which are omnipresent in most biological populations. In general they represent gene effects. However, they by no means represent the whole effects of the inheritance. Even in these data, sex differences are genotypic effects but are attributable to gene influences or their equivalent, transmitted generally as whole chromosomes, and developing their effects through internal balances throughout the whole organism. The component analyses of the different variances in Table III, with given embryological ages constant, are as shown below.

Source of variation	d.f.	Components of variation
Among genotypes	8	$E + 2_jG$
Among dosages	$(j - 1)$*	$E + 18T$
Genotype × dosage	$8(j - 1)$	$E + 2GT$
Between sexes	1	$E + 9_jF$
Sex × genotype	8	$E + jFG$
Sex × dosage	$(j - 1)$	$E + 9FT$
Sex × genotype × dosage	$8(j - 1)$	$E + FGT$
Unaccounted for variation		E

* $j = 3, 4$, or 5, depending on which embryological age is analyzed.

The components can be interpreted as follows: G is the variation due to genotypic or hereditary differences as partially measured by strain differences. T is the variation due to differences in effects of the dosage levels, and F is the genotypic variation induced by the sex differences in the lifespans under the conditions of irradiation. The interaction terms are interpreted as arising from the differential responses of the genotypes of sexes from one level of irradiation to the next. The term, E, is considered due to uncontrollable variation, and represents random variation of individual differences of mice of the same sex within a litter given the same treatment.

The chart at the top of Figure 8 gives the graphs showing the contributions of the different components of variance plotted on the embryological ages when the irradiations took place. Interactions of FG, FT and FGT are combined, as they are small, and designated I. A similar analysis of the variance contributions, where the constant element is the irradiation dose and the embryological age becomes a variable, is shown in the graphs at the bottom of the figure. Again the interactions FG, FU and FGU are combined and represented as I. Between these two charts an appreciation may be gained of dosage (T) and embryological effects (U) as well as those of genotype (G), and sex (F) and their interactions (I). The scale of the ordinates is percentage of total variation which is contributed by these various components.

The differences in the capacities of the two sexes (F) to receive irradiation at different stages in embryological development and later perform the functions normal to life are shown to have the most effect in altering mature lifespan for the mice under investigation. These effects hold whether the irradiations are at early, medium or later periods in embryological development. They hold for all dosages of radiant energy here under investigation as shown in the lower chart.

The next large contributor to variance under the conditions as shown in either chart is the variance made up of the effects of many factors, most of which, even in the best controlled environments, are still environmental in origin, E, the uncontrolled variance. This variance is most important in the early embryological stages and lower dosages of irradiation.

Genotypic influences on mature lifespans (G) are materially influenced by the time in embryological age and the level of irradiation dosage when they are considered. In the 6½ and 10½ embryological age groups the genotypic differences, as marked by the strains, have little effect on the later lifespans. Similarly, the strain differences show little effect if the dosages of irradiation are only 20 or 80 roentgens. The low values of these genotypic effects may be due to two causes. The anatomical and physiological structures of the embryos through 10½ days may not have differentiated the genetic elements, or the time sufficient for them to reach a size for radiant energy to produce apparent effects on life. Similarly, irradiation dosages of 20 and 80 r received in the embryological stages may be insufficient for them to affect processes significant to mature lifespan.

By 14½ days gestation the genotypic effects have begun to express themselves. They continue to show their effects in the embryos at 17½ days and in newborn mice. The effects are on the order of 10%. They become increasingly important to animals irradiated with larger dosages, increasing from 7% for the 160 r to 26% for 320 r treated mice.

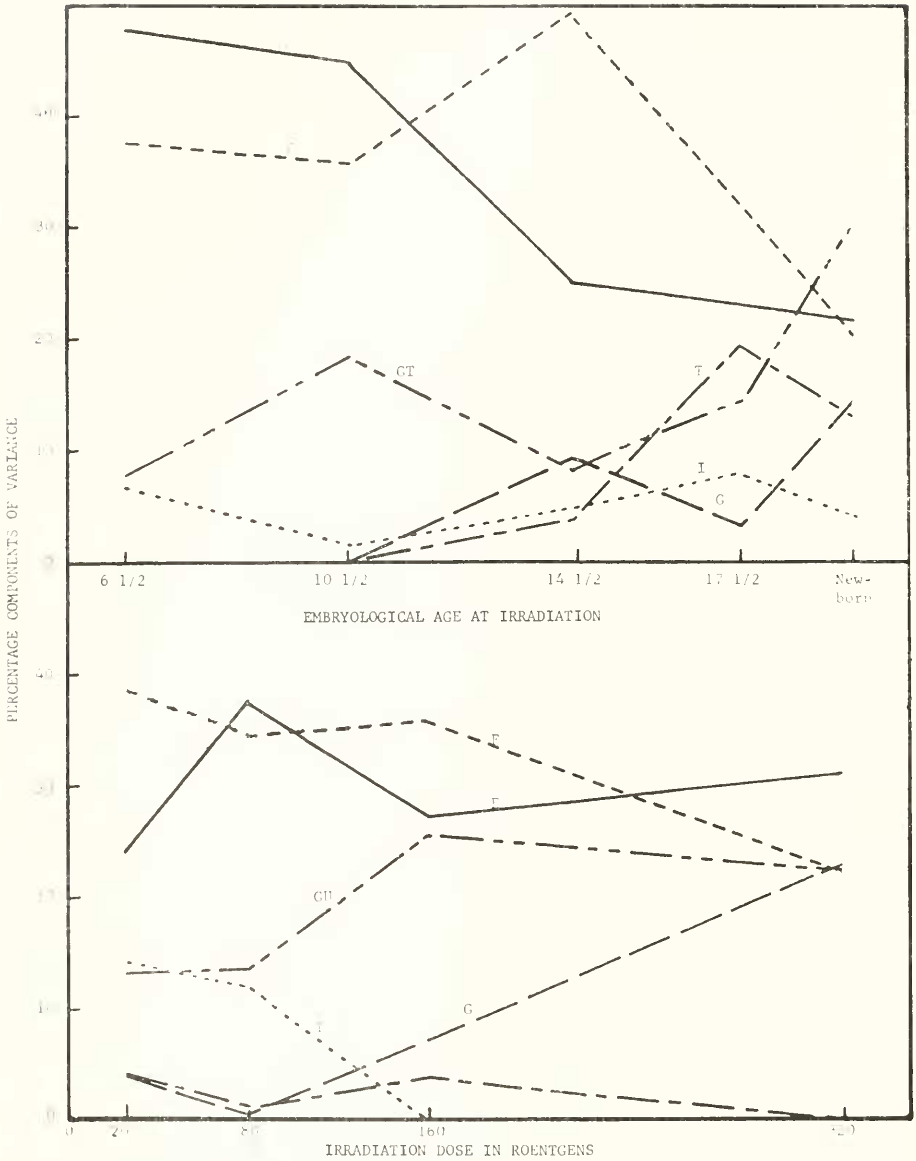

Figure 8. Contributions to variance of mature lifespan in these studies, upper chart, by genotypic and sex differences in strains of mice following *in utero* exposures to specific x-ray dosages received at different stages in embryological development. The effects of the different components are shown by the following lines; Genotype (G) — — —; x-ray dose (T) — - —; interaction (GT) — - - —; Sex (F) - - - -; combined sex interaction (I) . . . .; and unaccounted for variations generally of environmental origin (E) —. Lower chart, the variance evaluations are distributed on x-ray dose received; G, F, I and E have like significance to those shown by the same lines in the upper chart. The effect of stage in embryological development when irradiation was received (U) is shown by — - —; and the (GU) interaction effects by — - - —.

Genotype also interacts with both irradiation dosage (*GT*) and with stage in embryological development (*GU*). These contributions to variance range from 10% to 30%.

Individually the embryological stages when the irradiations took place have relatively small percentage effects on the mature lifespans, 0–5%, although they do have pronounced effects on the juvenile survival.

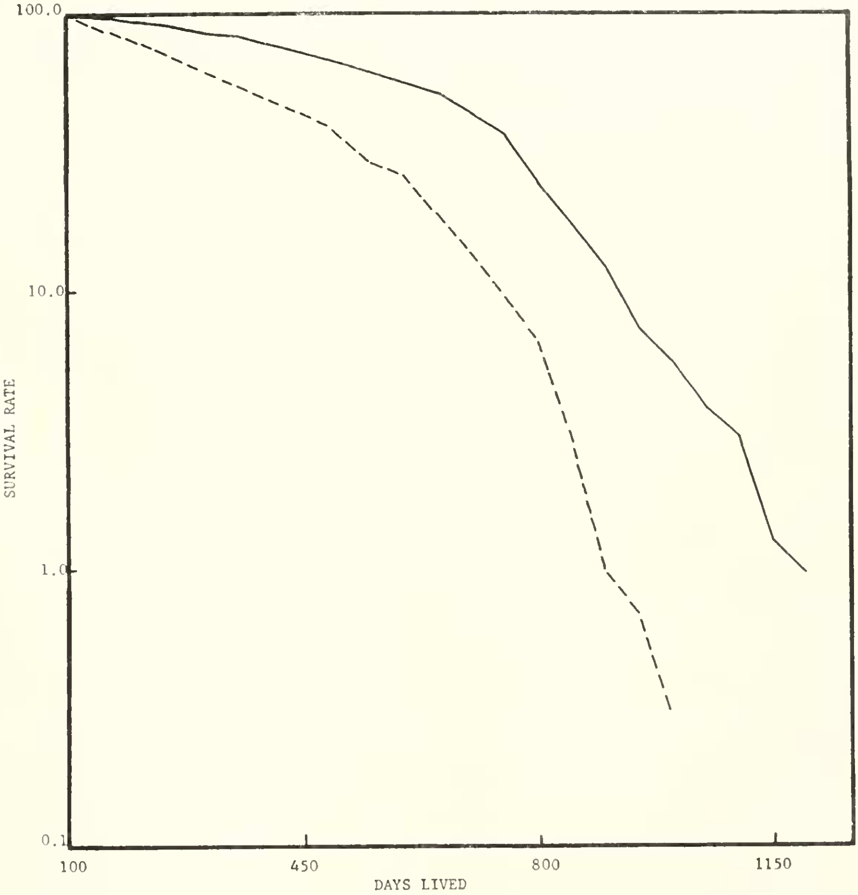

FIGURE 9. Survival rates of male and female mature mice exposed to various dosages of irradiation during uterine development. Males —, Females - - -.

There are three different interactions which include sex of the treated mouse in each of the different embryological or dosage treatment groups. Individually these effects are small and by statistical standards insignificant. These interactions are combined in the lines marked *I* on the charts. They approach uniform values throughout the different treatment groups. Their total contribution to variance range is between 0 and 15%.

Survival Curves of Mature Mice Following Embryonic Irradiation

The survival patterns of these mice may be brought out more fully in life curves showing the proportions of mice surviving at successive ages over their known life-spans. Figure 9 gives these data separately for the males and females in the populations. The numbers of males and females in each group are equal.

Male survival rates are at all ages greater than those of the females. At the stage when the population has reached only 1% of those which started as mature mice at 100 days, the males have lived 300 days more than the females. As pointed out in the earlier analyses the greater part of the survival rate differences of the sexes is attributable to physiological differences in the reproductive functions rather than to the irradiations. The form of the male survivals is like that of the females in showing noticeable curvature with age, indicating that no one agent is responsible for the life changes.

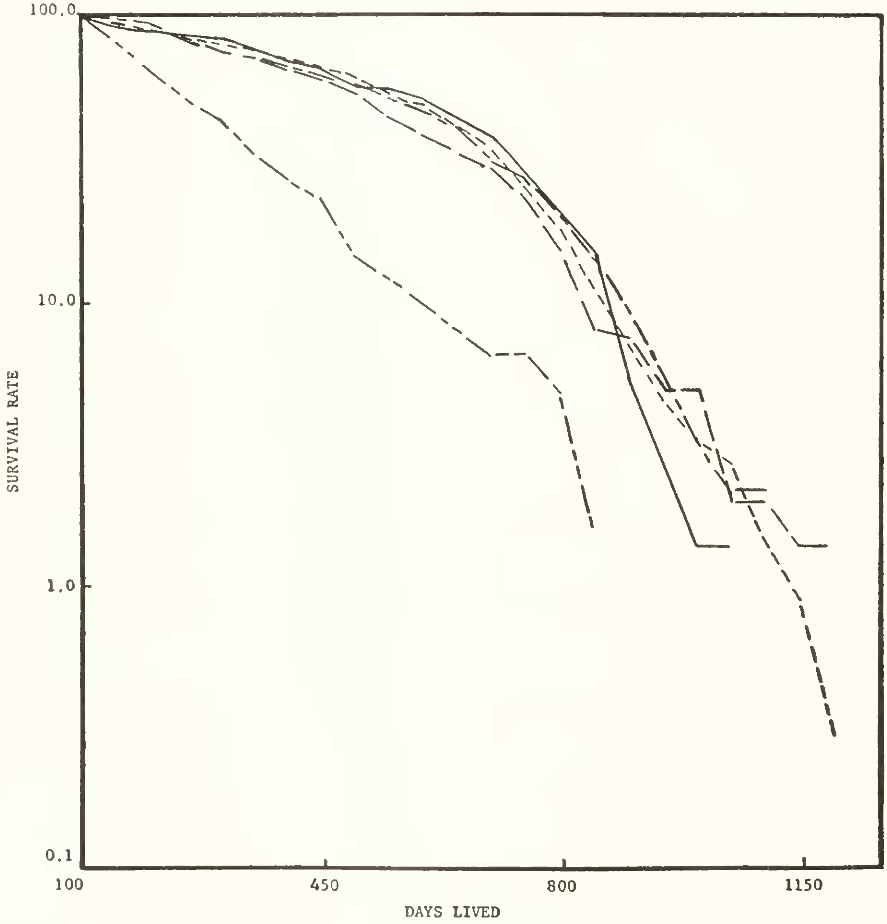

Figure 10. Survival rates of mature mice exposed *in utero* to different dosages of irradiation. Zero roentgens —, 20 r - - -, 80 r — - —, 160 r — —, and 320 r — - - —.

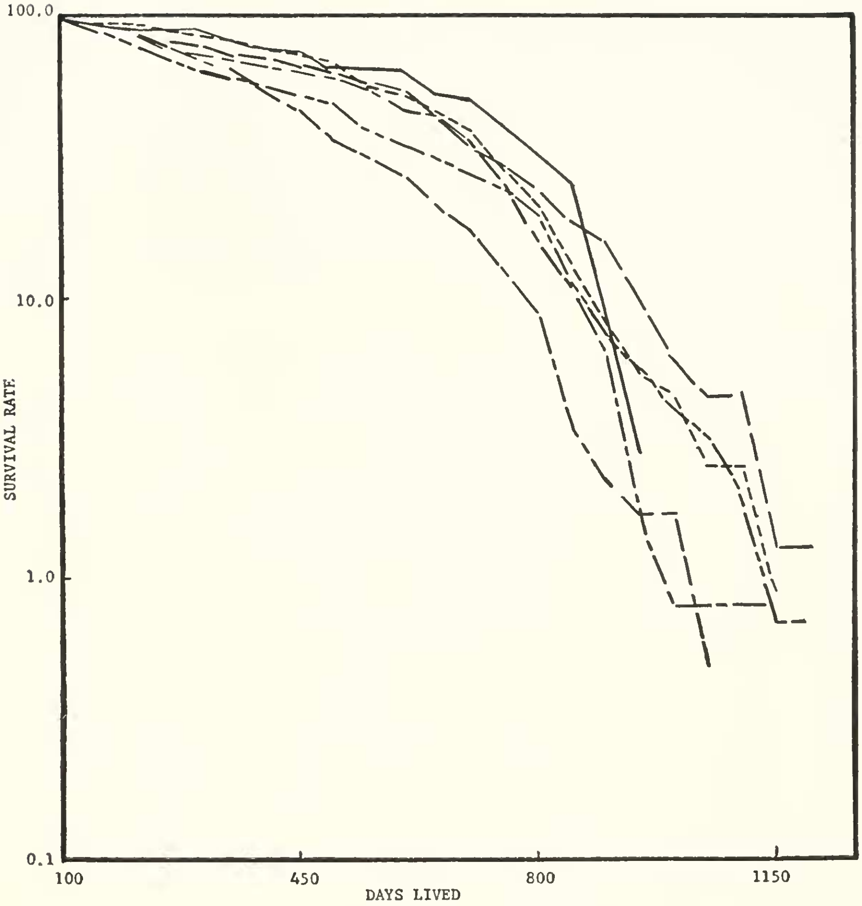

Figure 11. Survival rates of mice exposed to x-irradiation at different stages of uterine development Untreated —, 6½ days gestation - - -, 10½ days — —, 14½ days — - —, 17½ days — - - - —, and newborn — — - — —.

Figure 10 arranges the data to show the survival rates for the different x-ray dosages. For these data the sexes are balanced. Survival rates are shown for the 5 dosage treatments.

The survival rates for the 0, 20, 80 and 160 r are all smoothly flowing curves convex to the age axis for the mature mice. Deaths are few at the beginning of maturity. They increase and become more frequent as age advances. When 50 of the cohort of 100 have died, the survival rate differences are not large but they are in order of the x-ray dosages the mice received *in utero*. At 850 days of age the unirradiated control mice show a decrease in survival rate of unknown cause. In general, for those mice reaching adult age, the survival rates are fairly comparable in the range of x-ray dosages of 0 through 160 r.

Changes induced by 320 r irradiation while the mice are still *in utero* have permanent effects on survival even when the mice reach young adulthood. The rate of survival takes the form of a simple exponential instead of the convex form of the lesser dosages. Each day of life is accompanied by a constant percentage decrease in survival. The chart makes it obvious that above 160 r the effects of x-radiation on adult lifespan increase rapidly. For 160 r, 10% of mice remain alive at 835 days while with 320 r, 10% survival is attained at 600 days or 72% of the lifespan reached by the lower irradiated mice. The detrimental actions of 320 r x-ray acute irradiations resulted in pronounced lowering of adult lifespans. These losses in expected lifespan occurred even though the mice previously had passed through an earlier intense selection period eliminating those so susceptible to irradiation as to die *in utero* as in the juvenile period of development.

Figure 11 gives the survival curves of these mice distributed by the stage in embryological development when the x-raying took place. The curves cover a fair range although not as great as that observed for the extreme x-ray dosages. At the point where 50% of the mice in the original populations had died, the untreated mice had the longest survival; the 6½- and 10½-day treatments were next and about equal to each other; the 14½-day treatments showed slightly less survival, 17½-day noticeably less and the newborn least of all. The sequence of the survivals again comes from the fact that the severely detrimental actions of 320 r and 160 r at some embryological ages have destroyed the original orthogonal character of the experiment. For the 10½-day period only, the mice treated with 0, 20 and 80 r reached the beginning of the adult stage. Similar selective action occurs for some of the 6½- and 14½-day treated mice. Some irradiated mice can live for long periods.

In each irradiated group 1% to 7% of the group lived longer than the unirradiated controls of this study. All of the curves for the mature age lifespans for mice irradiated at the different embryological ages are convex to the age axis, indicating that the mouse characteristics operated on by the radiant energy are numerous and interacting.

DISCUSSION

Lifespan is a variable made up of many composite parts, each of which must integrate perfectly with the other, as well as the environmental factors, to bring it to its full expression. It is the variable which is most likely to measure quantitatively on a single scale the embryological, physiological and morphological damage accumulated by the exposed animal.

Somatic cells arising from the germ line have equal opportunities for genetic change. Without any wastage it takes some 43 cell generations (successive divisions) to develop a mouse. This number is beyond our grasp, 2^{43} for each individual. During these divisions there are many opportunities for gene mutations and chromosome aberrations to occur in the same way as they appear in the germ line.

Mutations notoriously require conditions of proper embryological development for their effects to receive phenotypic expression. Different thresholds of change appear throughout life but the right one must be reached for the appearance of characteristic effect of the given gene.

Aberrant phenotypes appearing in populations of mice receiving irradiation during embryo development likewise follow this step-like pattern. The aberrant phenotypes observed for the same radiation dosages given at different *in utero* stages show changes characteristic for expected stage of development when irradiation took place. Seldom do they show changes related to those naturally appearing in later development. The threshold pattern points to necessary specific conditions in both host and treatment for the appearance of the effect. The ultimate causes of the changes in physiomorphic development affecting lifespan seem best sought from this viewpoint, rather than in hypotheses generated through indistinguishable lumping of causative components into single constants to form one of the many curves which may be theoretically generated, *e.g.*, the Gompertz curve.

The direct effects of the irradiations on the exposed cell may be on the genes, the chromosomes, the action links between the genes and chromosomes, the cytoplasm, or cytoplasmic elements. With the large numbers of ion pairs released per roentgen it seems impossible to think that any cell often escapes receiving at least one and often many when irradiated with the large dosages usually employed. Yet the evidence shows that sometimes cells do perform their functions even when they have been exposed to high dosages of radiation. It appears that cells have escape mechanisms for reducing the customary irradiation damage which may be immediately effective, or effective after an interval of further development. There are several categories into which these radiation effects may be classified. The growth pattern of the cell may be so altered as to make its future cells function abnormally with regard to the organism as a whole. Other cells may stop cell division, restricting an organ system essential to the whole. Subsequent growth processes may be so ineffective that a new event must occur if the damage is to be repaired. In the latter category may be included some gene or chromosome aberration—generated phenotypes. Chromosome aberrations initiated by breakage, on the other hand, may rejoin and the cell recover its previous normal functions. Differential cell growth where the irradiation damage is "healed" through replacement of defective cells by others of normal constitution, as in wound healing, offers another means of arriving at a normal phenotype. However useful this mechanism is, it is not recovery of the irradiation-damaged cell but should be labelled one of cell replacement. Cytoplasmic elements affected by radiation frequently are not carried through future mitoses or are incapable of reorganization. They offer recovery mechanisms for their repair.

Data on all types of cells seem to indicate that the greater parts of their volumes are not occupied by radiation-sensitive structures. Rather they allow the radiation to pass through, or, when absorption occurs, are unaffected.

Earlier studies on malformations following ionizing radiations were concerned primarily with effects that were observed in embryos some time between the time of irradiation and shortly after parturition (Bagg, 1922; Job, Leibold and Fitzmaurice, 1953; L. B. Russell, 1950; W. L. Russell, 1954; O'Brien, 1956; and Rugh, 1953, 1959). The results generally agree that the type of malformation depends both on the level of irradiation and the embryological stage of development during which irradiation took place. Most exposures were to high dose acute irradiations causing cell damage far beyond the physiological limits the exposed animals might repair.

Additional studies in recent years have attempted to evaluate the long-term sequelae of embryonic or fetal irradiation. It has become evident from these investigations that although an animal exposed to embryonic irradiation may appear normal at birth, effects on morphological and behavioral characteristics may have been produced that become noticeable in later life, as cataracts (Stadler, 1959; Gowen, 1963) or volitional activity (Huff and Gowen, 1960). In some instances changes following embryonic irradiation have been found both by histological study and by functional tests of the organ system under study. Beaumont (1960, 1962), in quantitative histological studies of the ovary and testis in rats, demonstrated a deficiency in germ cells following embryonic irradiation. Several workers, including Rugh and Jackson (1958), Russell *et al.* (1959), and Nash and Gowen (1961), observed in mice long-term effects on the reproductive system in that adult reproductive capacity had been altered following embryonic irradiation.

Effects of prenatal irradiation upon postnatal growth have been reported for several species, including the mouse (Russell *et al.,* 1959; Nash and Gowen, 1962), the rat (Ershoff and Bavetta, 1958), and cattle (Parish *et al.,* 1962). In several instances effects of prenatal irradiation upon growth did not become evident until some time after treatment. Nash and Gowen (1962) noted, for example, that certain embryological treatments did not produce a noticeable effect upon body weight until some two months after treatment. Similar "delayed effects" also have been reported for certain behavioral effects. Rugh (1956) found that mouse embryos irradiated with doses of 5 to 300 r exhibited more nervous excitability than controls when tested at 15 days and two months of age. In some cases behavioral changes have been observed in later life although no distinct morphological malformation or lesion could be demonstrated. It is evident from these examples that there may exist changes induced by embryonic irradiation which may not have a noticeable effect until late in life.

Although ionizing radiations have been widely used as an experimental means to accelerate changes sometimes associated with aging in mammals (Gowen, 1962), the exact relationship between lifespan and other physical and biological variables is still not clear. Attention has been directed towards changes progressive with age, sex, genetic background, and radiation dose response. Abrams (1951) found that the effect of irradiation on survival decreased rapidly with increasing embryological age and increasing age after birth. Similar results were reported for the rat by Stearner and Christian (1951), who observed that radioresistance increased rapidly between one and 48 hours after birth so that by two days of age rats were only slightly less radioresistant than adults. Although some workers (Sacher, 1947, 1957; Blair, 1956) have postulated that radioresistance should probably decrease in some uniform manner with advancing age, other workers, including those in our laboratory, have found periods of relative stability of radioresistance. Kohn and Kallman (1956), for example, found that the $LD_{50/30}$ was a linear function of age from 37 to 105 days of age, but remained nearly constant from 115 to 709 days. Spalding and Trujillo (1962) noted in mice irradiated between two and 21 months that radioresistance was relatively stable through the first half of adult life but then declined quite rapidly. Sacher (1957) also found little dependence on age and mean accumulated dose to death of animals irradiated between 100 and 600 days.

There have been few systematic studies concerned with the effects of *in utero* irradiation upon adult lifespan. Zuikova *et al.* (1959) reported that in dogs irradiated during the last trimester of pregnancy, the duration of lifespan was in direct relation to dose. Russell *et al.* (1959) found that postnatal viability to 36 days of age in mice was lowered in animals irradiated with 200 r on days 9½, 7½, or 11½. In long-term survival, of the data available at that time, lifespan appeared reduced in those mice that had been exposed to 200 r on days 7½, 11½, or 13½.

Considering the four embryological stages used in this investigation, 6½, 10½, 14½, and 17½ days, and the levels of irradiation, through 160 r, it is difficult to discern clear-cut "critical periods" for the induction of changes in adult lifespan such as have been described for several other characteristics. Instead, noticeable effects on lifespan appear to be a result of interaction of a certain radiation dose with a certain specified embryological stage. The embryological stage at which embryos were irradiated did not seem to influence adult lifespan as long as the dosage did not exceed 80 roentgens.

At the higher dose level the exceptions occur in female mice treated at 14½ and 17½ days gestation. After 160 r exposures life shortening occurs at a more rapid rate than expected from the previous trend in lifespans observed in 0, 20, 80, 160 r treatments. These changes are indicative of a threshold effect coming in this region of dosage, initiated by the radiation absorptions occurring in a more resistant set of receptors as well as those already taking up the radiant energy.

The consequences of acute irradiations have generally been studied as changes closely following irradiation as expressed in different morphological types, morbidity and rapidity of death in 30 days. The data herein show that as far as duration of adult lifespan is concerned, the effects may be carried quiescent through to expression over a fairly wide age span in later life. In this sense the late effects become comparable with more limited characters such as cataracts following irradiation, but on a different time scale. The shortening of life is the consequence of the experience through which the mouse passed at some embryological stages of development. The cataract stimulation, although having a long quiescent period of 300+ days during which no changes were evident, was dose-dependent and results from acute irradiation dosages occurring at 46 days from the birth of the animal when most of the embryological pattern of development was complete (Gowen, 1962).

The nature and mode of action of the changes induced by embryonic irradiation that result in a shortening of the lifespan are still speculative. There is increasing evidence that aging may be caused by the gradual accumulation of spontaneous chromosomal rearrangements and mutations in the somatic cells of the body (Gowen, 1934, 1962; Curtis, 1963). Through several methods of controlling chromosome aberrations it has been shown that their frequency was inversely proportional to the life expectancy.

Teratogens are also capable of producing chromosomal anomalies in various tissues. Mustard gas acting on sperm of *Drosophila* furnishes one of the early examples (Crowe, 1961). Ingalls *et al.* (1963) demonstrated that a teratogen, 6-amino nicotinamide, when administered to pregnant female mice about the 13th day of gestation, produced chromosomal anomalies. Cells exhibiting polyploidy and fragmented chromosomes were observed not only from tissues adjacent to palatal defects but in tissues remote from the palate as well.

In the present study, embryonic x-irradiation offers a means of producing chromosomal aberrations in the developing embryos. Although some of the embryonic cells may harbor mutated chromosomes they may function in a normal or near-normal fashion. Others may cause marked changes in body maintenance with a subsequent curtailment of life. A wide range of change leading, however, to like as well as different effects, may be induced giving high variability even within progenies of given inbred litters.

A major concern of the present investigation was to evaluate the contribution of genetic background on lifespan in response to *in utero* irradiation. Although mammals, and the mouse in particular, have been widely used in radiation and aging studies, there is a surprising lack of information on lifespans in mice born of different mating systems. Inbred and hybrid mice, such as were used in this investigation, provide insight into the basic radiation responses over the period when aging is occurring. Several studies in the past have demonstrated that genetic constitution is a major factor in adult lifespan. Gowen and Stadler (1956) utilized 10 genetically differentiated strains of mice to study the effects of irradiation at 40 days of age upon lifespan. The study included the three strains reported in the present paper. Genetic differences were found to influence the effects of x-rays throughout the acute dose range up to 960 roentgens. Genotypic strain differences were observed by the expression of different radiation syndromes. Chai (1959) also observed genetic differences in lifespan. In general, hybrid mice had longer mean life and lower mortality at earlier ages than those of the parental inbred strains. This may be due to the hybrids having a greater reserve of cells unaffected by such environmental agents as radiation on which to draw for life maintenance (Haverland and Gowen, 1960).

Differences among inbred strains considered to be primarily genetic in origin, have been noted by Henshaw (1944), Grahn (1954), Gowen and Stadler (1956), Haverland and Gowen (1960), Kohn and Kallman (1957), and Grahn and Hamilton (1957). Progress has been made towards understanding the nature of the genetic and physiological factors conditioning the radiation response. Kohn and Kallman (1956) reported that in the strains of mice employed by them, greater sensitivity was a result of recessive genetic factors. Grahn (1958), in crosses involving the least and most resistant strains from a series of lines, estimated the genetic mean of the LD_{50} to be about 50% of the total variance. Presumed single-gene differences in radiation response have been observed by Doolittle (1961) and Bernstein (1962).

Stadler and Gowen (1957a, 1957b, 1957c) studied the effect of three body regions on radiation response in five inbred strains of mice, as measured by percentage survival and length of survival. Differences between responses of strains were noted at different levels of irradiation. The extent of the interactions among x-ray doses and strains was large, indicating that each strain had a distinctive pattern of radiation response.

In the results of the effects of embryonic irradiation upon lifespan reported in the present paper, a quantitative examination of genetic influences was provided by a variance analysis and a breakdown of the variance into its component parts. The variance analysis revealed that strain differences influenced the length of adult life when embryos were exposed to irradiation during the latter part of gestation, from 14½ days on. This would indicate that by this stage in development genetic differences have been established sufficiently well in the mouse so that irradiation produces differential responses, as measured by adult lifespan. There are indications from other studies (Rugh, 1958, 1963; Nash and Gowen, 1963) that genetical differentiation, as measured by radiation response, may be expressed even earlier in embryological development. The lack of significant strain differences in the dosage sequences following irradiation at 6½ and 10½ days is a reflection of the toxicity of lower acute x-ray dosages received by these strains. When the embryos have passed these thresholds in development the influence of strain differences on subsequent life becomes less.

Of particular interest in the present data is the presence of significant dose-by-strain interactions following irradiation late in pregnancy. This indicates that the different genotypes are responding through different physiological channels over the range of radiation levels. Interactions of this sort following embryonic irradiation were also reported for postnatal growth by Nash and Gowen (1962). In the present study genotype-by-treatment interaction accounted for up to 30% of the total variation in adult lifespan. The existence of this type of interaction furnishes an excellent tool for further understanding of the basic radiation response.

The possible existence of genetically determined variation in the timing of embryological development, as indicated by this analysis, lends biological significance to the mechanisms of radiation-induced lifespan changes. The early work of Painter (1928) and Venge (1950) demonstrated that differences in size between Flemish-giant and small Polish rabbits could be traced back to early embryonic stages and differences in cleavage rates of fertilized ova between large- and small-sized breeds. Mechanisms having these qualities offer means of understanding how they and other genetic differences may appear in radiation effects on later lifespan and other effects.

The evidence from Nash and Gowen (1962) shows that these mice irradiated *in utero* were reduced in size following irradiation. The growth reductions were dependent on both dose and stage in embryological differentiation. The weight changes to 75 days were small for the 20 and 80 r treated mice. They became more severe at 160 r, particularly for the 10½-day irradiations where at birth the mice were but ⅔ size and unable to survive to the 12-day age. The 75-day weights were consistently reduced following 320 r irradiation.

These changes can be related to observations on like actions by other agents which result in the separation of blastomeres, or cutting of Planaria (Morgan, 1898) into small pieces which on regeneration result in separate animals of small size. The x-ray action seems to come from reducing the numbers of certain vital cells. It is as though there was, for each egg type, a limit to the total cells which may divide. In eggs where the blastomeres lose their totipotence, the loss becomes that of specific cells, precursors of specific organs. In this case these organs may be affected, giving the appearance of specificity to the abnormalities observed in mice irradiated at fixed stages of development in the embryological sequence.

Schaible (1959, 1963) has studied the sequence of melanocyte distribution in mice and chickens of different genotypes. These cells distribute to eight centers of the mouse coat. Whiteness can be the result of absence, malfunction or death of the pigment cells. The distribution of the pigment leaves a record of the events through which the cells have passed in developing their final products. In general, changes in pigment cell density within the normal sequence do not influence organizing cells to promote cell death. The products are left to reveal patterns of organogenesis. The observations become more complete than for other cells as in the organization of the nervous system where damage or loss of neuroblasts (Rugh, 1962) may remain obscure for lack of visible signs.

The outward spread of pigment from eight centers is revealed in three ways: pigment spots on unpigmented background; absence of pigmented cells in a pigmented background; and the presence of a spot of different-colored hair in the coat, representing the descendant cells of a primordial melanoblast which had changed to a genotype different from that of the original zygote from which it started. The pattern of melanocyte descent offers possibilities of control of the end products of development by both genetic and environmental factors. The factor interactions furnish a significant model for the variations observed spontaneously and under x-radiation in the lifespan of this study. The coat color model displays differences between inbred strains of different genotypes ranging from zero to 90%. Substitution of different genes gives a staircase type of ascent to the frequencies of change within the mouse populations. Genotypic species changes are marked by even broader differences in the timing of the periods when hair or feather follicles may attract melanocytes: in the mouse all follicles of the coat seem to mature at one time and exhaust the epidermal supply of melanoblasts; in the chicken the epidermal supply of melanoblasts does not exhaust, at least in the growing animal.

In this model, tests of the pigment mosaics show they are, in large majority, somatic rather than germinal, agreeing with the evidence on the lifespans of mice under continuing low rate irradiation (Stadler and Gowen, 1963; Gowen and Stadler, 1964). There were no whole-body reversions of coat color in 5165 offspring nor did wild type segregates on test have unexpected gametes. Changes appear to occur only after at least one primordial pigment cell has established.

X-radiation with 150 r to the region of the gravid uterus from $\frac{1}{2}$ to $11\frac{1}{2}$ days, following copulation, in day intervals, revealed two significant high points in mosaic frequencies of the progeny, 2.5 and 10.5 days *in utero* development. However, the acute dose administered had only slight influence in the total mosaics observed over the full period of the irradiations. The suggestions of periods of embryological development of greater susceptibility to acute x-ray dosages, and the lack of pronounced changes in frequencies of mosaics from the unexposed controls, again model what was observed in our data on survival in after-life for the lower dosages of acute x-ray irradiations.

SUMMARY

1. Mature lifespans, 75 days of age to death, when the mice were exposed to irradiations at different stages of uterine development, showed reductions in mean longevity which were dependent on both x-ray dose and period of embryological cycle. The design for this study was factorial. There were five irradiation

treatments, 0, 20, 80, 160 and 320 r, six embryological stages: untreated, 6½, 10½, 14½, 17½ and newborn (19½) days of uterine development, two sexes and nine mouse strains. Three of the inheritance groups were inbred strains and six were the reciprocal crosses of these strains. Efforts were made to obtain two mice for each cell of the design. However, some treatments were so severe that this was impossible. X-ray dosages of 160 and 320 r at embryological ages 6½, 10½ and 14½ days were too lethal to obtain the requisite number of progeny in these groups.

2. Six hundred and forty-seven completed mouse lives were collected. Mature lifespan ranged downward from 1194 days for the males and 921 days for the females. Both of these long-lived individuals had been irradiated, the male with 160 r at 14½ days embryological development and the female with 80 r at 10½ days embryological development. The frequency distributions of lifespan were of several types for the different groups, depending on the treatments, strains or sexes of the individuals.

3. In terms of changes in mature lifespan, sex differences in the mice resulted in marked differences in the mean days they survived. Considering the *in utero* treated mice, the average unirradiated females survived for only 69% of the mean male lifespan. This male-to-female difference cannot be attributed to irradiation effects but rather to the hazards which differentially affect the physiological processes which separate the lives of the sexes, chiefly those associated with reproduction. Adjusting for these effects shows that the average female life under irradiation still exhibits greater sensitivities to irradiation *in utero*. Considering the available data for the dosages taken separately, the 20 r females had 85% of the mean lifespan observed for the untreated females, the males 96.7% that of the untreated males. Like comparisons for the 80 r were 82 vs. 93; for the 160 r, 73 vs. 89 and for the 320 r, 30 vs. 43.

4. Intrauterine irradiations, when limited to 20 to 80 r, reduced the female durations of life at all stages of embryological development more than they did those of the males.

5. Variance analyses emphasized the importance of sex as a controlling element in adult survival when the mice are under pressure of reproduction.

6. Genotypic differences, as measured by strain and as separate from sex, have little effect on subsequent mature lifespans when the irradiation is small in amount, 20 including 80 r, or when given at 6½ or 10½ days of embryological development. The amount of radiant energy absorbed becomes more important to mature life's duration when the dosages of radiation are larger, 160 including 320 r, or when the exposures occur in the latter half of pregnancy. The variance analyses support the significance of the differences on which the above conclusions were based.

7. Genotype also interacts with both stage of embryological development when irradiation occurred and with the dosage of x-rays absorbed.

8. Individually the embryological stages when the irradiations took place have but small percentage effects on mature lifespan, although they do have pronounced effects on survival in the juvenile stage. All changes in lifespan come as direct irradiation effect to the soma of the embryos, as caused by acute irradiation. Lifespan lowering comes largely in the 320 r treated embryos in the

form of a threshold effect which then carries through to the adult as cryptic damage to appear later in life.

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